

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
FENAZAQUIN [designated as Technical EL-436 in many reports]

Chemical Code # 6029, Document Processing Number (DPN) # 53114

8/23/2011

Revised date (Not applicable)

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	Data gap, inadequate study, no adverse effect indicated
Reproduction, rat:	No data gap, no adverse effect
Developmental toxicity, rat:	No data gap, no adverse effect
Developmental toxicity, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers for the above study types through 258405 (Document No. 53114-0090) were examined. This includes all relevant studies indexed by DPR as of 8/23/2011.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: t20110823.wpd

Original by Aldous, 8/23/11. Revised by Name, Date (Not applicable)

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

COMBINED, RAT

**53114-0061 253028 Cocke, P. J., P. C. Francis, S. M. Boss, V. P. Meador, and C. L. Gries, "A chronic/oncogenic toxicity study in Fischer 344 rats administered EL-436 (Compound 193136) in the diet for 2 years," Lilly Research Laboratories, Greenfield, IN, 4/24/92. Laboratory Study # RC5253. Sixty F-344 rats/sex/group were dosed for 2 yrs in diet with EL-436 (fenazaquin), Lot ACD13041, purity 98.5% at 0, 10, 100, 200, or 400 (M)/450 (F) ppm. Achieved dose levels were 0.5, 4.5, 9.2, and 18.3 mg/kg/day in respective treated males, and 0.6, 5.7, 11.5, and 25.9 mg/kg/day in corresponding females. Absolute NOEL = 10 ppm (slight decrement in cholesterol and very slight decrement in triglyceride levels, particularly in females). These do not appear to be candidates for critical endpoints. Consistent, dose-related changes at 200 ppm and above include body weight and food consumption decrements in addition to consistent and often substantial decrements in cholesterol (each of these changes in both sexes). These changes support 100 ppm as a functional NOEL. Body weights were reduced 16-18% at 400-450 ppm, clearly defining an MTD. Gross and microscopic pathology found no treatment-related effects. Acceptable, with no adverse effects. Aldous, 3/14/11.

CHRONIC TOXICITY, RAT

See Combined, Rat (above).

CHRONIC TOXICITY, DOG

**53114-0056 253023 Cosse, P. F., K. E. Stebbins, R. J. McGuirk, J. R. Ormand, and H. S. Stewart, "XDE-436: 1-year dietary toxicity study in beagle dogs," The Dow Chemical Co., Midland, MI, 5/14/93. Laboratory Study # DR-0316-5240-003. Groups of 4 beagle dogs/sex/group were dosed in diet with XDE-436 (Fenazaquin), Lot # 435MH8, 98.1% purity, for 1 year in a chronic study. Dose levels began at 0, 1, 5, and 15 mg/kg/day (dietary concentrations based on ongoing food consumption and body weight measurements), however the highest dose was reduced to 10 mg/kg/day after 13 weeks, due to substantial body weight decrements (1.4 kg in M and 1.3 kg in F at day 93) associated with decreased food consumption at 15 mg/kg/day. As a result, the highest dose level averaged 12 mg/kg/day, and is so designated in the report. High dose males had reduced cholesterol levels compared to pre-test and concurrent control levels. This was plausibly related to reduced food consumption. NOEL = 5 mg/kg/day, based on diminished body weights and diminished food consumption in both sexes, and on reduced cholesterol in males. Acceptable, with no adverse effects. Aldous, 3/11/11.

ONCOGENICITY, RAT

See Combined, Rat (above).

ONCOGENICITY, MOUSE (HAMSTER STUDY WAS SUBMITTED FOR THIS PURPOSE)

53114-0057 253024 Francis, P. C., S. M. Boss, and C. L. Gries, "A carcinogenicity study in Syrian golden hamsters administered EL-436 (compound 193136) orally for 18 months," Lilly Research Laboratories, Greenfield, IN, 12/22/92. Laboratory Study # HC0307. Groups of eighty Syrian golden hamsters/sex/group (100/sex/group in controls) were gavage dosed (5 ml/kg, in 10% aq. acacia vehicle) with EL-436 (fenazaquin), Lot #ACD13041, purity 97.28%, for 18 months in an oncogenicity study. Dose levels were 2, 15, and 30 mg/kg/day in treated males, and 2, 15, and 35 mg/kg/day in treated females. NOEL = 2 mg/kg/day, based on statistically significant body weight decrements in both sexes (note that body weight decrements in 15 mg/kg/day females were quite modest, although statistically significant for 12 weeks of the study). There was a significant **reduction** at 15 mg/kg/day and above in both sexes of "severe" grade kidney amyloidosis, and a significant reduction in amyloidosis as presumptive "cause of death" at these dose levels in both sexes. The study is not acceptable. The mouse is a much better characterized model than the Syrian golden hamster, and the reasons presented to seek an alternative to the mouse (i.e. that the mouse is comparatively insensitive to test article, in part due to rapid metabolism) were not compelling. Compared to the mouse, the hamster has a comparatively low background level of tumors, with only adrenal cortical cell and thyroid c-cell tumors showing incidences over 1-2% in this study. Hepatocellular tumors, often the most sensitive tumor response in mice, were entirely absent. Thus this species is likely to be insensitive to treatment-induced tumors. Survival was skewed in favor of the two highest dose groups of each sex, associated with markedly lower incidence and/or degree of amyloidosis, a common life-limiting process in this species. Thus only the lowest dose level behaved physiologically like controls. The study was compromised by a major *Clostridium* infection, which required antibiotic treatment for the latter half of the study. The infection caused deaths of many animals, including 15 high dose males and 20 high dose females, and manifested itself as inflammatory changes in the intestinal tract in many survivors. This set of issues indicates too many uncontrolled variables for a robust oncogenicity study, and should be classified as **unacceptable, and not upgradeable**. There was a statistical increase in adrenocortical tumors in high dose females compared to concurrent controls. This appears to be incidental considering that (1) that high dose females had much higher survival than controls, (2) that this tumor type was only observed at or near to scheduled terminal sacrifice in females in this study, (3) that the incidence and degree of adrenocortical amyloidosis was inversely dose-related, possibly rendering control group survivors less likely than concurrent controls to such tumors, and (4) there were no elevations of pre-disposing lesions in the adrenal cortex. A separate submission (DPR Document No. 53114-0059, Record No. 253026) provided historical control data on adrenocortical tumors. Cited studies found an overall mean of 6.5% adenomas and of 1.7% adenocarcinomas in control females. Thus incidences observed in the present study (adenomas at 2.0, 2.5, 7.5, and 10.0% in controls through increasing dose groups of females, and corresponding adenocarcinoma incidences of 0, 0, 2.5, and 2.5%) were close to historical incidences for the test species. No adverse effect is indicated. Aldous, March 11, 2011.

53114-0060 253027 Francis, P. C., "EL-436 (compound 193136): experimental miticide: justification for use of the hamster in an oncogenicity study," 7/17/90. The author justifies use of hamster instead of mouse for the 2nd species for oncogenicity studies, primarily because (1) hamster is the more sensitive species to the most consistent indicator of toxicity (body weight depression), and (2) hamster shows dose-independent kinetics, whereas the mouse has impaired clearance at high doses. The author noted that mice displayed about 7 x increases in peroxisomal β -oxidation at limiting dose levels, whereas rats experienced about 2 x increases,

compared to no significant increase in hamsters: the implication being that testing the 2 species with similar hepatic response levels would be redundant. Having concluded that the hamster is the best 2nd rodent species for oncogenicity evaluation, the author noted two design features which appeared best for a lifetime hamster study: use of shoebox cages with bedding to reduce stresses possibly associated with wire-bottomed caging, and use of gavage treatment for adequate assessment of achieved dose, due to the hamster's habit of storing much of food offered. Useful supplementary information. Since this was not a report of a study, but a justification for species selection based on various data, no detailed worksheet is relevant. Aldous, 3/16/11.

NOTE: 53114-0089 258404 is an exact copy of Record No. 253027, above.

53114-0080 258395 Ernst, H., I. Kunstyr, S. Rittinghausen, and U. Mohr, "Spontaneous tumours of the European hamster (*Cricetus cricetus* L.)," Z. Versuchstierkunde 1989; 32: 87-96. This article describes observations on hamsters derived from northern Germany, and compares and contrasts this stock with related Chinese and Syrian hamsters. These European hamsters live substantially longer than Syrian hamsters, and have different neoplasm patterns, hence chronic disease features of one of these species would not be very predictive of the other. Useful supplementary information. No DPR worksheet. Aldous, 7/13/11.

53114-0058 253025 Stott, W. T., U. Vedula, D. M. Bond, and K. E. Stebbins, "Potential of XDE-436 analogues to induce hepatic hypertrophy and peroxisome acyl-CoA oxidase activity in mice," The Dow Chemical Co., Midland, MI, 2/18/93. Laboratory Study # T2.02-201-000-001. Groups of female CD-1 mice were dosed daily by gavage with 150, 300, or 600 mg/kg/day fenazaquin for 4 days in a study to assess (1) peroxisome acyl-CoA oxidase activity induction, (2) liver weight effects, and (3) liver histopathology. A series of analogs and metabolites was evaluated for comparison, typically at comparable molar dose levels. Specific peroxisome acyl-CoA oxidase activity due to fenazaquin was elevated with dose-response over this range, with 600 mg/kg/day group activity elevated over controls by 2.5 x to 5.1 x over several assays. Some metabolites also showed elevated peroxisome acyl-CoA oxidase activity, particularly the ether cleavage product (*t*-butyl-benzylethanol) and an oxidation product of the *t*-butyl moiety (*t*-butyrate analog). Some test articles were much more toxic than fenazaquin. Most materials increased liver weights, but typically less than fenazaquin. In general, materials resulting from ether cleavage elicited hepatocellular hypertrophy to a similar extent as fenazaquin, whereas oxidation products of the *t*-butyl moiety elicited less hypertrophy than fenazaquin. Useful supplementary data. Aldous, March 11, 2011.

53114-0090 258405 This is a memo from U.S. EPA dated 5/15/07. Subject was "Fenazaquin: PP# 9E5059. Tolerances on apples, pears and citrus fruits exported to the US. HED Risk Assessment. PC Code: 044501, Decision #: 302678, DP #: 325204. Page 9 of the memo acknowledged the supplementary report justifying the use of golden Syrian hamsters (i.e. Record No. 253027 above), without comment as to some of the concerns stated in the DPR review (see study 53114-0057 253024, above). This study was rated by U.S. EPA as "Acceptable/Guideline." The review acknowledged the enteritis problems and the need to mediate with antibiotics (p. 44), noting evidence of systemic toxicity and survival issues. The decision to accept the study despite some enumerated problems was also "supported by Senior Cancer Experts at a meeting on March 22, 2007." The U.S. EPA memo did not address issues of differential survival or of the great reduction of "severe" grade kidney amyloidosis at the two

higher dose levels, each of which was a concern stated in the DPR review. No DPR worksheet, since this is a U.S. EPA review, not a study. Aldous, 7/13/11.

Data Evaluation Record, Fenazaquin, OPTS 870.4200, "Study Type: Carcinogenicity Study - Hamster," MRID No. 45029913 with supplements MRID 43798702 and 44742910, primary U.S. EPA reviewer: Dana F. Glass. A copy of this DER was submitted by the registrant to DPR in response to DPR concerns about the above mouse oncogenicity study (DPR Document No. 53114-0057, Record No. 253024). Key conclusions of this DER were summarized in the above U.S. EPA Risk Assessment Record No. 258405, above. Three main concerns raised in the DPR review were not satisfactorily addressed in the U.S. EPA review, in particular: (1) choice of the test species, (2) acceptability of the study despite a major infection process, and (3) skewed survival. This DER relates the reasons given by investigators for using the hamster as a test species, primarily its increased "sensitivity:" the hamster having a much lower NOEL than the mouse. The DER acknowledged the magnitude of the *Clostridium* infection with its associated mortalities, and accepted the investigators' argument that antibiotic treatment maintained sufficient animals long enough for a valid study. The DER noted that survival in female controls and lowest (2 mg/kg/day) dose group was remarkably lower than in higher treatment groups, and lower than the normally acceptable level of 25% for a rodent lifetime study (18-month treatment period), but added that survival at 17 months was within those guidelines. This DPR reviewer found no new or compelling arguments for accepting this study. DPR has accepted some studies in hamster instead of the mouse, but reasons for use of the hamster have to be weighted against the low background incidence of tumors in hamsters, with the associated low likelihood of showing significant tumor increases due to treatment. The many premature deaths and confounding effects of antibiotic treatment associated with *Clostridium* infection greatly weaken the tenuous argument for hamster as surrogate for the mouse: the mouse rarely presents so devastating of a disease process in laboratory studies. Survival in females strongly favored the highest two dose groups. This resulted largely from reduction in those groups of the degree of amyloidosis, a common life-limiting process in this species. Thus only the lowest dose level behaved physiologically like controls. In summary, the DER does not provide any new reasons to accept this study. Aldous, 7/22/11 (no DPR review, since this DER is not a study).

53114-0081 258396 Francis, P. C. This supplement provided additional historical incidence of adrenocortical tumors in Syrian golden hamsters. These data support the original DPR assessment that this tumor type does not appear to have increased due to treatment in the hamster oncogenicity study. Useful supplementary information. No DPR worksheet. Aldous, 7/13/11.

53114-0079 258394 Boss, S. M., C. L. Gries, B. K. Kirchner, G. D. Smith, and P. C. Francis, "Use of vancomycin hydrochloride for treatment of *Clostridium difficile* enteritis in Syrian hamsters," *Laboratory Animal Science* **44**: 31-37 (1994). This is a publication reporting the investigators' experiences in treating hamsters in the primary fenazaquin hamster oncogenicity study (53114-0057 253024, above). Since the essential information was included in the oncogenicity study report, no DPR worksheet is needed. Aldous, 7/13/11.

REPRODUCTION, RAT

**53114-0055 253022 Christian, M. S., R. M. Hoar, and A. M. Hoberman, "Reproductive effects of EL-436 (Compound 193136) administered orally via gavage to Crl:CD® (SD)BR rats

for two generations with one litter per generation,” Argus Research Laboratories, Inc., Horsham, PA, 8/9/91. Laboratory Study # Argus 112-002. Groups of at least 30 CrI:CD® (SD)BR rats/sex/group/generation were dosed daily by gavage with EL-436 (fenazaquin), Lot 271MH8, purity 98.4%, in a 2-generation reproduction study at 0, 1, 5, and 25 mg/kg/day. The most suitable parental systemic toxicity NOEL = 5 mg/kg/day, based on modest body weight decrements during pre-mating periods. Clinical signs of “excess salivation” were evident at all dose levels: sharply dose-related. These signs usually began after at least 14 days into the study, and were observed sporadically for any given rat, and with frequencies which varied much between rats of a given dosage group. Salivation did not impact reproductive viability. Parental reproductive effects NOEL = 25 mg/kg/day (highest dose tested). Offspring viability and growth NOEL = 25 mg/kg/day (highest dose tested). Acceptable. No adverse effects. Aldous, 3/15/11.

53114-0054 253021 Christian, M. S., T. Martin, and A. M. Hoberman, “Reproductive effects of EL-436 (Compound 193136) administered orally via gavage to CrI:CD® (SD)BR rats for two generations, with one litter per generation,” Argus Research Laboratories, Inc., Horsham, PA, 7/30/92. Laboratory Study # Argus 112-003. Groups of 30 CrI:CD® (SD)BR rats/sex/group were dosed by gavage daily throughout the study with EL-436 (fenazaquin), Lot 271MH8, purity 98.4% in a reproduction study at 0 and 40 mg/kg/day. This is a supplementary study to the main reproduction study (DPR Document No. 53114-0055 253022), and was designed to identify high dose effects. Parental systemic toxicity NOEL < 40 mg/kg/day, based body weight decrements and clinical signs of “excess salivation” in both sexes, and on “reduced motor activity” in F1 males. Parental reproductive effects NOEL = 40 mg/kg/day (highest dose tested). Offspring viability and growth NOEL < 40 mg/kg/day, based on a 6-8 g body weight decrement of 21-day weanlings, and a plausibly treatment-related increase in F2 stillborn pups. Valid supplementary information. Aldous, 3/15/11.

Overall NOEL’s from the above two studies may be summarized as follows. A toxicologically important parental systemic toxicity NOEL = 5 mg/kg/day, based on modest body weight decrements during pre-mating periods. Note that “excessive salivation” was dose-related in incidence and frequency at all dose levels tested (1 to 40 mg/kg/day), without evident functional sequelae. Parental reproductive effects NOEL = 40 mg/kg/day (highest dose tested). Offspring viability and growth NOEL = 25 mg/kg/day, based on a 6-8 g body weight decrement of F2 21-day weanlings, and a plausibly treatment-related increase in F2 stillborn pups at 40 mg/kg/day. Aldous, 3/15/11.

DEVELOPMENTAL TOXICITY, RAT

**53114-0053 253020 Francis, P. C., and G. L. Higdon, “A teratology study of EL-436 (Compound 193136) administered by gavage to CD rats,” Lilly Research Laboratories, Greenfield, IN, Aug. 10, 1989. Laboratory Study # R08989. Groups of 25 SD rats/sex/group were dosed by gavage in 10% aq. acacia solution with EL-436 (fenazaquin), purity 98%, Lot ACD13041, at 0, 3, 10 or 40 mg/kg/day over gestation days 6-17. Maternal toxicity NOEL = 10 mg/kg/day (food consumption decrements throughout the dosing period at 40 mg/kg/day, with associated body weight gain decrements). Developmental toxicity NOEL = 40 mg/kg/day (highest dose tested). Acceptable, with no adverse effects. Aldous, 3/15/11.

DEVELOPMENTAL TOXICITY, RABBIT

****53114-0052 253019** Francis, P. C., and G. L. Higdon, "A teratology study of EL-436 (Compound 193136) administered by gavage to New Zealand White rabbits," Lilly Research Laboratories, Greenfield, IN, 4/24/92. Laboratory Study # B02289. Twenty NZW does/group were dosed by gavage with 0, 3, 13, or 60 mg/kg/day EL-436 (fenazaquin), Lot ACD13041, purity 98%, on gestation days 6 through 18 in a developmental toxicity study. Maternal toxicity NOEL = 13 mg/kg/day (modest food consumption decrement on study days 6-12 at 60 mg/kg/day). Developmental toxicity NOEL = 13 mg/kg/day (indicated by three high dose does with total early resorptions: in each case with only 1-2 implantations). There was no treatment effect on body weights of pups nor were malformations nor variations associated with treatment. The high dose provided only 8 viable litters, compared to 15 in the middle dose group and 16 in controls. Except for the noted early resorptions, losses in high dose litters were apparently not associated with treatment. There were no treatment-related malformations, deviations, or variations. While this study would have benefitted from more closely spaced dose levels in the range of an MTD, available data suggest it unlikely that another dose level between 13 and 60 mg/kg/day would have found remarkable effects. This study is acceptable, despite the noted problems of the 60 mg/kg/day group. No adverse effects are indicated. Aldous, 3/15/11.

GENE MUTATION

****53114-0088 258403** Francis, P. C., J. C. Scheuring, and K. K. Richardson, "The effect of EL-436 (Compound 193136) on the induction of reverse mutations in Salmonella typhimurium and Escherichia coli using the Ames test," Lilly Research Laboratories, Greenfield, IN, 10/31/89, Laboratory Study Nos. 890327AMT2884 and 890814AMS2884. Tests were done in triplicate, with 187.5, 375, 750, 1500, or 3000 µg/plate fenazaquin [EL-436, Lot ACD13041, purity 98%], plus negative controls and positive controls (at two levels for each strain). Five strains (TA 1535, TA 100, TA 1537, TA 98, and *E. coli*: WP2 uvrA⁻) were exposed to various concentrations of fenazaquin for 48 hrs at 37 °C. Fenazaquin was not cytotoxic at the limit test of 5000 µg/plate: the highest dose had been selected based on precipitation at 4000 µg/plate and above. Study is negative for mutagenicity. Acceptable. Aldous, 8/23/11.

****53114-0087 258402** Francis, P. C., K. K. Richardson, and K. C. Michaelis, "The effect of EL-436 (Compound 193136) on the induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells," Lilly Research Laboratories, Greenfield, IN, Nov. 12, 1989. Laboratory Study Nos. 890719MLT2884, 890726MLA2884, and 890816MLA2884 (for the toxicity test, and for two complete assays, respectively). Mouse lymphoma cell L5178Y sub-line TK^{+/-} cultures were assessed in a forward mutation assay at closely-spaced doses, limited by cytotoxicity with and without S-9 at about 10 µg/ml. There was no mutagenic response without S-9 activation. The first trial with S-9 found 3x or greater mutation indices at 6, 8, and 12 µg/ml. Of these only 6 µg/ml was within acceptable range of survival (19%), whereas higher dose levels had survival ≤ 6%. Due to the apparent positive response, the test with S-9 was repeated with two additional trials. High dose response of increased mutation index was repeated in both cases at 6 µg/ml and above. In each of the latter cases, survival at 6 µg/ml was > 10%, hence considered valid. Investigators justifiably classified test material positive with S-9 activation. Acceptable, with a "possible adverse effect." Aldous, 8/23/11.

CHROMOSOME EFFECTS

**53114-0086 258401 Francis, P. C., M. L. Garriott, and D. E. F. Kindig, "The effect of EL-436 (Compound 193136) on the *in vitro* induction of chromosome aberrations in Chinese hamster ovary cells," Lilly Research Laboratories, Greenfield, IN, Nov. 8, 1989. Laboratory Study Nos. 890725CTX2884, 890802CAB2884, and 890816CAB2884. Chinese hamster ovary cells (sub-line WB_L) were evaluated with EL-436 (fenazaquin), Lot ACD13041, purity 98% at 0, 0.1, 0.5, and 1 µg/ml without S-9 activation, and at 0, 40, 50, and 60 µg/ml with S-9. Selected highest dose levels were founded on cytotoxicity (21% survival without S-9, and 52% survival with S-9). Positive controls, mitomycin C without S-9 and cyclophosphamide with S-9, were functional. Study is acceptable, and is negative for chromosomal aberrations. Aldous, 7/25/11.

53114-0084 258399 Francis, P. C., M. L. Garriott, and J. D. Brunny, "The effect of EL-436 (Compound 193136) on the *in vivo* induction of sister chromatid exchange in bone marrow of male CD-1 mice," Lilly Research Laboratories, Greenfield, IN, 11/22/89, Laboratory Study Nos. 890911ATX2884 and 890926SCE2884. Three male CD-1 mice/group were dosed by gavage with 0, 500, 1000, or 2000 mg/kg EL-436 (fenazaquin), Lot ACD13041, purity 98%. Tablets containing 20-30 mg BrdUrd were implanted subcutaneously 5 hrs before administration. Colchicine (4 mg/kg) was administered 19 hrs after treatment, and mice were sacrificed 2 hrs later. Femur bone marrow cells were obtained, fixed, washed, dried, then stained with Hoechst 33258 followed by Giemsa to create a long-lasting stain. Twenty-five metaphases were counted per mouse. Cytotoxicity was evaluated by examining 100 metaphases for first, second, or third division staining characteristics. The study did not show increased SCE's, but had too few experimental units for proper analysis. Surviving fenazaquin-treated mice consisted of 3 at 500 mg/kg, 2 at 1000 mg/kg, and 1 at 2000 mg/kg. Fenazaquin did not systematically slow cell cycle. Positive controls were functional. Unacceptable. Aldous, 7/27/11.

**53114-0083 258398 Francis, P. C., J. W. Parton, and M. L. Garriott, "The effect of EL-436 (Compound 193136) on the *in vivo* induction of micronuclei in bone marrow of ICR mice," Lilly Research Laboratories, Greenfield, IN, 9/22/89. Laboratory Study Nos. 890718ATX2884 and 890725MNT2884. Groups of 5 ICR mice were dosed twice with EL-436 (fenazaquin), Lot ACD13041, purity 98% by gavage at 24-hr intervals, with harvest of femur marrow cells 24 hrs after the last treatment. Fenazaquin groups received 0, 400, 800, and 1600 mg/kg/day for males, and 0, 400, 800, and 1200 mg/kg/day for females. Respective highest dose levels were about 50% of the single gavage dose LD₅₀ for fenazaquin. Positive controls received 40 mg/kg cyclophosphamide on the above schedule. PCE to NCE ratios did not vary systematically between negative controls and fenazaquin treated groups. Micronuclei were markedly elevated after cyclophosphamide treatments, but not after any of the fenazaquin treatments. Acceptable, and negative for micronucleus response. Aldous, 8/17/11.

DNA DAMAGE

**53114-0085 258400 Francis, P. C., M. L. Garriott, and D. J. Yount, "The effect of EL-436 (Compound 193136) on the induction of unscheduled DNA synthesis in primary cultures of adult rat hepatocytes," Lilly Research Laboratories, Greenfield, IN, 10/18/89. Laboratory Study Nos. 890516UDS2884 and 890718UDS2884. EL-436 (fenazaquin), Lot ACD13041, purity 98% was evaluated for UDS potential in two independent trials, with hepatocytes from different male F-

344 rats. Exposure of attached cells was 20 hrs. Readable fenazaquin concentrations were 0.001, 0.005, 0.01, 0.05, and 0.1 µg/ml in trial one and 0.001, 0.005, 0.01, 0.05, 0.1, and 0.5 µg/ml in trial two; usable concentrations in both trials limited by cytotoxicity. Positive controls were 2-acetylaminofluorene (2-AAF) at readable levels of 0.05, 0.1, and 0.5 µg/ml (activation required for mutagenic expression), and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) at readable levels of 1 and 5 µg/ml (activation not required for mutagenic expression). Each trial had 4 replicate negative controls. All fenazaquin treatments had net negative grain counts, with no large or consistent differences from untreated controls. Positive controls were clearly functional. Acceptable, negative for UDS. Aldous, 7/25/11.

NEUROTOXICITY

Not required at this time.

METABOLISM

53114-0082 258397 Althaus, W. A., and J. D. Magnussen, "Disposition and metabolism of orally administered ¹⁴C-EL-436 in Fischer 344 rats," Lilly Research Laboratories, Greenfield, IN, May 1, 1992. Laboratory Study Nos. R18289, R31489, R37189, and ABC-0449. Groups of usually 5 F-344 rats/sex were dosed by gavage with EL-436 (fenazaquin), Lot ACD13041, in a metabolism study. Purity of unlabeled fenazaquin was 97.4 to 98.8%. Radiochemical purities of labeled materials were 99.2% for quinazoline-phenyl ring and 97.3-97.7% for t-butyl phenyl ring label. Investigators used 1:1 mixtures of the 2 labeled materials by specific activity. Treatments were single dose at 1 or 30 mg/kg, or repeated dose with unlabeled fenazaquin for 14 days, followed by labeled dose at 1 mg/kg. Investigators evaluated urinary and fecal metabolites for 7 days, at which time major tissues were evaluated for residues. Initial study found no significant ¹⁴C-CO₂ in exhaled air. Irrespective of treatment regimen, about 20% of administered dose was excreted in urine in either sex, and about 80% was excreted in feces. Most of label was excreted within 24 hrs for urine and within 48 hrs for feces. Regardless of dosing regimen, about 1% of administered dose remained in carcass 7 days after treatment. Concentration in fat exceeded concentration in any other tissue, typically by a large margin. In females, concentration in ovaries was higher than any other organ or tissue except fat. Only one urinary metabolite was characterized, a product of hydrolysis of the ether linkage: (2-hydroxy-1,1-dimethylethyl) phenylacetic acid, about 25% of urinary residues. An additional 20-25% of urinary residues was evidently composed of unidentified neutral unconjugated metabolites. Ten to 15% of urinary residues appeared to conjugates, which yielded neutral aglycones upon enzymatic hydrolysis. Feces contained small amounts of parent following 1 mg/kg dose (1-4% of fecal radioactivity). High dose administration led to fecal excretion of 12-21% of fecal residues at parent, suggesting impaired absorption. The most common characterized metabolite in feces involved oxidation of a methyl group of the t-butyl moiety to the carboxylic acid (about 20% of fecal label). The same metabolite, with additional hydroxylation on the quinazoline group, accounted for an additional 11-13% of fecal label. The only other common fecal metabolite was parent with hydroxylation on a t-butyl methyl (5-9% of fecal label). Study is not acceptable. Only about 25% of urinary metabolite residues was characterized, and less than 50% of fecal residues was characterized. Also, since about 80% of labeled residues appeared in feces, a means to assess absorbed dose (such as biliary cannulation study) should have been done. Pharmacokinetics aspects were adequately assessed. Aldous, 8/19/11.

SUBCHRONIC (and subacute, if applicable)

****53114-0047 253014** Cocke, P. J., P. C. Francis, and C. L. Gries, "A subchronic toxicity study in Fischer 344 rats given EL-436 (Compound 193136) in the diet for 3 months," Lilly Research Laboratories, Greenfield, IN, March 4, 1992. Laboratory Study # R27388. Groups of 10 rats/sex/group were dosed in diet with EL-436 (fenazaquin), Lot 271MH8, purity 98.4%, for 3 months at 0, 15, 45, 150, or 450 ppm. Estimated mean achieved dosages in treated males were 1.0, 3.0, 9.6, and 28.7 mg/kg/day, with corresponding females receiving 1.2, 3.5, 11.5, and 33.0 mg/kg/day. Absolute NOEL, based on liver enzyme induction of *p*-nitroanisole O-demethylase activity, is 15 ppm (males) and 45 ppm (females). In addition, benzphetamine N-demethylase activities were elevated in both sexes at 150 to 450 ppm, with associated NOEL's of 45 ppm for both sexes. A NOEL for toxicity (exclusive of palatability and liver induction) is 150 ppm, based on significantly elevated serum ALT, AST, and LDH activities in males. Additional findings likely associated with altered liver function include reduced cholesterol in 450 ppm males, and reduced globulin at 450 ppm in both sexes. Significant overall food consumption reductions were evident in both sexes at 450 ppm, probably associated with significant final body weight decrements of 46 g in males and 13 g in females. In addition, 150 ppm females showed significantly reduced food consumption for the first 4 study weeks. Study is acceptable, with no adverse effects. Aldous, 3/14/11.

53114-0048 253015 Francis, P. C., V. N. Ward, and C. L. Gries, "A subchronic toxicity study in Fischer 344 rats treated orally with EL-436 (Compound 193136) for 3 months followed by a 1-month reversibility period," Lilly Research Laboratories, Greenfield, IN, April 8, 1992. Laboratory Study # R12188. Groups of 15 rats/sex/group were dosed by gavage with EL-436 (fenazaquin), Lot N85-JX1-70, purity 99.4% , for 3 months in a supplementary subchronic study at 0, 1, 3, 10, and 30 mg/kg/day. An additional 10/sex were dosed for 3 months at 0 and 30 mg/kg/day and were maintained off treatment for 4 wks to assess recovery. Absolute NOEL, based on liver enzyme induction of *p*-nitroanisole O-demethylase activity, is < 1 mg/kg/day (males) and 10 mg/kg/day (females). A NOEL for parameters typically assessed in subchronic studies would be 3 mg/kg/day in both sexes, based on factors primarily indicative of liver responses. Liver relative weights were increased in 30 mg/kg/day males and liver absolute and relative weights were increased in 10 and 30 mg/kg/day females. Cholesterol levels were significantly reduced in 10 and 30 mg/kg/day males, and in 30 mg/kg/day females. There were small reductions in plasma proteins, particularly reduction of globulin in 30 mg/kg/day females, which might relate to altered liver function. There were dose-related increases in absolute and relative adrenal weights in both sexes at 10 and 30 mg/kg/day. There were no compound-related gross nor microscopic changes evident in any tissues. Significant overall food consumption reductions were evident in both sexes at 30 mg/kg/day, and there were significant final body weight decrements of 37 g in males and 12 g in females. Useful supplementary study, with no adverse effects. Aldous, 3/14/11.

53114-0049 253016 Francis, P. C., S. M. Boss, and C. L. Gries, "A subchronic toxicity study in Syrian golden hamsters treated orally with EL-436 (compound 193136) for 3 months," Lilly Research Laboratories, Greenfield, IN, March 4, 1992. Laboratory Study # H00190. Groups of 15 hamsters per sex per group were dosed with 97.6% fenazaquin [EL-436, Lot ACD13041] by gavage at 0, 5, 25, 75, or 150 mg/kg/day (M) or 0, 5, 25, 50, or 100 mg/kg/day (F) for 3 months. This brief DPR review summarizes key findings. There were no treatment-related deaths nor clinical signs. Progressive body weight decrements occurred in the two highest dose groups of

each sex: highly significant at termination. Final male body weight gain was 41, 49, 34, 19, and 11 g at increasing dose levels, compared to female gains of 74, 86, 72, 45, and 29 g. There was no sustained decrement in food consumption in either sex. There were modest decrements in hemoglobin at the highest two dose levels in both sexes. Clinical chemistry changes of reduced glucose levels (75-150 mg/kg/day males), cholesterol (males at 75 and 150 mg/kg/day, females at 25-150 mg/kg/day), and globulin (highest dose levels tested in both sexes) were among changes similar to findings of rat studies. Liver enzyme induction of *p*-nitroanisole O-demethylase activity was significantly elevated at and above 75 mg/kg/day (M) and 25 mg/kg/day (F). Liver relative weights were elevated at and above 75 mg/kg/day in males, as were liver and kidney relative weights in 50 and 100 mg/kg/day females. Absolute testes weights were significantly reduced at 75-150 mg/kg/day. "Severe" testicular atrophy was dose-related at these dose levels. This study shows 75 and 50 mg/kg/day to be non-sustainable dose levels for M and F, respectively. Useful supplementary data. Aldous, 3/14/11.

**53114-0050 253017 Cosse, P. F., K. E. Stebbins, H. S. Stewart, and C. N. Peck, "XDE-436: 13-week dietary toxicity study in beagle dogs," The Dow Chemical Co., Midland, MI, April 3, 1992. Laboratory Study # DR-0316-5240-002. Groups of 4 beagle dogs/sex/group were dosed in diet for 13 weeks with XDE-436 (Fenazaquin), Lot # 435MH8, 98.1% purity, in a subchronic study at 0, 1, 5, and 15 mg/kg/day (dietary concentrations based on ongoing food consumption and body weight measurements). NOEL = 5 mg/kg/day, based on an average of 22% reduction in food consumption for males and females during the first 16 days of the study, and periodic reductions thereafter: resulting body weight decrements were over 1.2 kg for males and 1.0 kg for females at termination. Additionally, decreased vacuolation of hepatocytes was observed in both sexes at 15 mg/kg/day, presumed to relate to the decreased food intake. Study is acceptable, with no adverse effects. Aldous, 3/14/11.

53114-0051 253018 Stott, W. T., P. F. Cosse, K. E. Stebbins, C. N. Peck, and N. L. Freshour, "XDE-436: palatability probe and two-week repeated dosing toxicity study in beagle dogs," The Dow Chemical Co., Midland, MI, 5/30/91. Laboratory Study # DR-0316-5240-001. The palatability study included 2/sex. Males were dosed with 5 or 10 mg/kg/day, with no definitive effects on food consumption. A female given 20 mg/kg/day had greatly decreased food consumption (averaging less than 25% of normal consumption). Another female on a graded dose regimen consumed variable amounts at 10 to 20 mg/kg/day, then less than 10% of normal at 40 mg/kg/day. These results showed that palatability was limiting at ≥ 20 mg/kg/day. The 2-wk repeated dose study involving 2 dogs/sex/dose at up to 10 mg/kg/day found no obvious effects on food consumption, body weights, or organ weights (only liver and kidney assessed). The latter study found no apparent effects at necropsy nor on histopathology. Probe studies support dose levels used in subchronic and chronic studies. Aldous, Jan. 4, 2011. No worksheet.